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The role of heat shock protein 27 and 60 levels in the follow-up of thalassemia patients

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Abstract

Aim: It was aimed to measure the levels of the heat shock proteins 27 and 60 as mediators to show hypoxia and tissue injury in thalassemia major and thalassemia intermedia patients followed in our clinic.

Material and Methods: 53 patients who had the diagnosis of thalassemia major (n=38) and thalassemia intermediate (n=15) and who presented to the Department of Pediatric Hematology at Gaziantep University were evaluated. 50 healthy children aged between 1-16 attending to general pediatric examination for routine control were taken as a control group (n=50). The relationship between clinical findings and laboratory parameters, and HSP 27 and HSP 60 were evaluated.

Results: The mean age was 9.2 ± 4.4 years in thalassemia major patients, 5.6 ± 3.8 years in thalassemia intermediate patients and 9.1 ± 3.7 years in control group patients. The level of HSP 27 was 5 ± 3 in thalassemia major, 4.2 ± 1.7 in thalassemia intermediate, 0.93 ± 0.76 in control group and the difference between patients and control group was significant ($p<0.05$). When the splenectomy status and HSP levels of the patients were compared, HSP 27 level was 7.5 ± 5 in patients who had undertaken splenectomy and was 3.7 ± 2.4 in patients who had not undertaken splenectomy and it was statistically significant ($p<0.05$). HSP 60 levels were 13.6 ± 20 in patients who had undertaken splenectomy and were 8.3 ± 32.4 in patients who had not undertaken splenectomy and it was statistically significant.

Discussion: Measuring HSP 27 and HSP 60 levels could be used as evaluation criteria in following of thalassemia major and in thalassemia intermediate patients the evaluation of complications.

Keywords

Anemia, Children, Heat Shock Protein, Thalassemia

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Introduction

Heat shock proteins are primarily involved in the transport, folding, binding and degradation of intracellular polypeptides, called chaperones, assembly and separation of oligomeric proteins within the cell [1, 2]. Some molecular chaperones attach to unfolded proteins, preventing them from being degraded and forming protein aggregates (for example: HSP70, HSP40). Some molecular chaperones capture damaged protein structures and ensure that these proteins are broken down with the help of chemical energy (for example: HSP60), that is, as a chaperone, HSP ensures that cell proteins are present in the right way, in the right place, at the right time [3, 4].

High HSP levels are only seen during the initial stages of stress. Even if the cell continues to be exposed to high temperatures after the initial shock, HSP levels begin to decline and return to normal levels. As a result, HSP contributes to the cell's adaptation to stressful conditions [5, 6].

HSP27 is located in both the cytoplasm and nucleus. In addition to thermotolerance, this protein plays a role in regulating the differentiation of epithelial cells, migration of keratinocytes during wound healing, preventing apoptosis and protecting cells from the cytotoxicity of inflammatory mediators. It also has a limited ability to protect enzymes against heat denaturation. HSP27 prevents apoptosis by inhibiting caspase activation, regulating the signaling pathway, or acting as a chaperone to protect cells [7].

HSP60, adhesion molecule E-selection, ICAM-1 and VCAM-1 expression from vascular endothelial cells; It also induces IL-6 release from vascular endothelial cells, muscle cells and macrophages. It is a protein known to be present in inflammatory events [8].

Thalassemias are autosomal recessive hemolytic anemias that occur due to the inability to produce one or more of the globin chains in the structure of hemoglobin in erythroblasts or to produce them in small amounts. Beta thalassemia, which presents with varying degrees of anemia, is the result of the inability to synthesize the chain required for adult hemoglobin as a result of mutation of the beta globin gene on the short arm of chromosome 11. Since alpha globin chains, which must combine with β globin chains, cannot form sufficient tetramers, they cause hemolysis by collapsing in erythrocyte precursors and erythrocytes [9].

Anemias generally progress with tissue hypoxia. Hypoxic conditions lead to increased synthesis of heat shock proteins (HSP) [10].

Especially in recent years, it has been reported that ischemia and thrombosis are more common in β thalassemia intermedia patients. A previous study found a 25% rate of silent cerebral infarction. In addition, iron accumulation occurs due to transfusions in thalassemia patients, and this iron causes damage to many organs such as the heart, liver and pancreas. Many parameters such as ferritin, oxidative stress indicators, antioxidant capacity, liver and heart MRI and T2 star measurements have been previously studied to evaluate tissue damage in thalassemia patients [11].

In this study, the importance of heat shock proteins as a new mediator in detecting tissue damage caused by hypoxia and iron accumulation in thalassemia major and thalassemia intermedia patients was discussed.

Material and Methods

Our study started in Gaziantep University Faculty of Medicine Hospital, Department of Child Health and Diseases, in accordance with the Declaration of Helsinki Decisions, Patient Rights Regulation and ethical rules, after the approval of the local ethics committee, Gaziantep University Ethics Committee, dated 01.10.2013 and numbered 01.10.2013/336. Additionally, the study was supported by Gaziantep University Scientific Research Projects Commission.

In this study, 53 patients who applied to Gaziantep University Pediatric Hematology Clinic and were diagnosed with Thalassemia major and Thalassemia intermedia were evaluated. Fifty healthy children, aged between 1 and 16 years, who applied to the general pediatric clinic for routine check-up, were included as the control group. The study and control groups were composed of subjects with similar age and gender. The patients were diagnosed with β-thalassemia by complete blood count, peripheral smear findings, maternal and paternal hemogram parameters, hemoglobin electrophoresis, and in some cases, molecular genetic analysis.

The files of the cases were examined retrospectively and their ages, gender, physical examination findings, laboratory parameters, abdominal ultrasonography and echocardiography findings were recorded and analyzed. HSP 27 and HSP 60 levels were read at 450 nm with an ELISA reader (Biotek Instruments, USA).

Statistical analysis

Kolmogorov Smirnov test was used to check the suitability of continuous variables for normal distribution. Student's t test was used to compare variables with normal distribution between 2 independent groups, and Mann Whitney U Test was used for variables with non-normal distribution. Kruskal Wallis test and Dunn multiple comparison tests were used to compare more than 2 independent groups. The relationship between categorical variables was tested with chi-square analysis. Descriptive statistics are frequency, percentage and mean ± std. deviation values are given. SPSS for Windows version 22.0 package program was used for statistical analysis and $P<0.05$ was considered statistically significant.

Ethical Approval

This study was approved by the Ethics Committee of Gaziantep University University (Date: 2013-10-01, No: 01.10.2013/336).

Results

In this study, HSP 27 and HSP 60 levels were studied in a total of 103 children, including 38 thalassemia major, 15 thalassemia intermedia and 50 healthy control groups. The relationship between HSP levels and clinical and laboratory findings was investigated. Among the individuals included in the study, there were 19 females and 19 males in thalassemia major patients, 7 females and 8 males in thalassemia intermedia patients, and 23 females and 27 males in the control group. There was no statistically significant difference in terms of gender in all 3 groups ($p = 0.90$).

The average age of thalassemia major patients was 9.2 ± 4.4 years, the average age of thalassemia intermedia patients was

Table 1. Comparison of HSP-27 and HSP-60 levels of cases

Groups	HSP 27 *Mean. ± SD	HSP 60 *Mean. ± SD
Thalassemia major	5 ± 3.9	10.1 ± 28.5
Thalassemia intermedia	4.2 ± 1.7	2.7 ± 3.1
Control	0.93 ± 0.76	0.35 ± 0.49
p value	0.01	0.01

* Mean ± Standard Deviation

Table 2. Comparison of the relationship between splenectomy status and HSP 60 and HSP 27 levels in patients with thalassemia major

Groups	Splenectomy + * Mean. ± SD	Splenectomy – * Mean. ± SD	p value
Thalassemia major HSP 27	7.5 ± 5	3.7 ± 2.4	0.016
Thalassemia major HSP 60	13.6 ± 20	8.3 ± 32	0.038

* Mean ± Standard Deviation

5.6 ± 3.8 years, and the average age of the control group was 9.1 ± 3.7 years.

HSP 27 level was 5 ± 3.9 in thalassemia major, 4.2 ± 1.7 in thalassemia intermediary, and 0.93 ± 0.76 in the control group. Accordingly, there was a statistically significant difference between the thalassemia major, thalassemia minor and control groups (p = 0.01). When the groups were evaluated separately among themselves, the following values were found for thalassemia major and thalassemia intermedia (p1-2 = 0.10), for thalassemia major and control group (p1-3 = 0.001), and for thalassemia intermedia (p2-3 = 0.001). HSP 60 level was 10.1 ± 28.5 in thalassemia major, 2.7 ± 3.1 in thalassemia intermediary, and 0.35 ± 0.49 in the control group. Accordingly, there was a statistically significant difference between the thalassemia major, thalassemia minor and control groups (p = 0.01) (Table 1). When the groups were evaluated separately among themselves in terms of HSP 60, it was determined for thalassemia major and thalassemia intermedia (p1-2 = 0.10), for thalassemia major and control group (p1-3 = 0.001), and for thalassemia intermedia (p2-3 = 0.001) (Table 1).

There was a statistically significant difference in platelet counts between the thalassemia major and control groups (p = 0.015). However, there was no statistically significant difference in platelet counts between thalassemia major, thalassemia intermedia and the control group (p > 0.05).

While splenectomy was performed in 13 of 38 patients with thalassemia major among the cases included in the study, there were no patients with splenectomy in the thalassemia intermedia and control groups. When the patients' splenectomy status and HSP values were compared, the HSP 27 value was 7.5 ± 5 in the splenectomy group and 3.7 ± 2.4 in the non-splenectomy group (p = 0.016). The HSP 60 value was 13.6 ± 20 in the splenectomy group and 8.3 ± 32 in the non-splenectomy group (p = 0.038). Accordingly, HSP 27 and HSP 60 were statistically significantly higher in patients who underwent splenectomy (Table 2.).

Discussion

In our study, there were a total of 103 cases: 38 with thalassemia major, 15 with thalassemia intermedia and 50 in the healthy control group. Considering the cases, the K/E ratio was 7/8 in thalassemia intermedia patients, 19/19 in thalassemia major patients, and 23/27 in the control group. Accordingly, in our study, no statistically significant difference was found in terms of gender in all three groups. According to the literature, the F/M ratio is generally reported to be equal in thalassemia major and thalassemia intermedia patients [12]. When the average age of all individuals included in the study was examined, it was found to be 9.2 ± 4.4 years in thalassemia major patients, 5.6 ± 3.8 years in thalassemia intermedia patients, and 9.1 ± 3.7 years in the control group, and it was noted that the average age of thalassemia intermedia patients was younger.

Studies have been conducted evaluating the relationship between iron load and oxidative stress in thalassemia major patients. In their study on 56 thalassemia major pediatric patients, Fatima et al. showed that the substance, which is an indicator of lipid peroxide, increased compared to the control group, and the determinants related to the antioxidant system decreased significantly [13]. According to this result, they suggested that non-transferrin-bound iron in serum and the intracellular iron pool induce peroxidative damage.

Heat shock proteins are molecular chaperones that assist in protein folding and translocation. The term molecular chaperone is defined as the ability of stress proteins to bind to cellular proteins to aid their transport or migration. Under cellular stress, proteins denature and can form aggregates. This event will ultimately result in cell death. Heat shock proteins bind to cellular proteins and protect them from aggregation during cell stress. The degradant helps heal damaged cells by binding to poorly folded polypeptides. It has been observed by various studies that heat shock proteins are induced by physiological thermal changes similar to fever. The fact that fever uses HSPs in important pathways that protect the organism against infection and other disease conditions and interacts with these pathways suggests that fever and HSPs developmentally protect the organism in the long term [14, 15]. Anemias generally progress with tissue hypoxia. Hypoxic conditions lead to increased synthesis of heat shock proteins [16]. Especially in recent years, it has been reported that ischemia and thrombosis are frequently seen in thalassemia intermedia patients. In a recent study conducted in patients with thalassemia intermedia, silent cerebral infarction was detected in a rate of 25% [17]. Again, tissue hypoxia generally occurs in thalassemia major patients, and this research aims to investigate whether HSP 27 and HSP 60 levels of thalassemia major and thalassemia intermedia patients can be measured and used as biomarkers in follow-up [17].

There was a statistically significant difference in platelet counts between the thalassemia major and control groups, and no statistically significant difference in platelet counts was detected between the thalassemia major, thalassemia intermedia and control groups. However, we found a slightly positive and significant correlation between platelet counts and HSP 27 and HSP 60 levels in thalassemia major patients. Consistent with the information in the literature [18], we found a statistically significant difference between the ferritin levels of thalassemia major and thalassemia intermedia patients

and the control group. A study found that markers related to oxidative damage showed a strong correlation with ferritin [19]. Based on this information, we expected a positive correlation between ferritin elevation and heat shock proteins. On the contrary, in our study, we did not find a statistically significant relationship between ferritin levels and HSP 27 and HSP 60 levels in the thalassemia major, thalassemia intermedia and control groups.

In our study, 13 of 38 patients with thalassemia major underwent splenectomy, while there were no patients with splenectomy in the thalassemia intermedia and control groups. When the patients' splenectomy status and HSP values were compared, the HSP 27 value was found to be 7.5 ± 5 in the splenectomy group and 3.7 ± 2.4 in the non-splenectomy group. The HSP 60 value was found to be 13.6 ± 20 in the splenectomy group and 8.3 ± 32 in the non-splenectomy group. Accordingly, we found HSP 27 and HSP 60 levels to be statistically significantly higher in patients who underwent splenectomy. Eldor et al. showed that there is platelet activation in both thalassemia major and thalassemia intermedia and that this condition is unrelated to splenectomy [20]. In a prospective study of 563 patients who underwent splenectomy in a study conducted in the Netherlands, portal vein thrombosis was found to develop in 9 patients (2%) and it was stated that these were mostly patients with hemolytic anemia [21]. In our study, the high values of HSP 27 and HSP 60 in patients with splenectomy can be speculated as splenectomy in thalassemia major has a negative effect on oxidative stress and tissue damage.

When the HSP 27 level of the cases was examined, it was found to be 5 ± 3.9 in thalassemia major, 4.2 ± 1.7 in thalassemia intermediary, and 0.93 ± 0.76 in the control group. According to this; HSP 27 levels of thalassemia major and thalassemia intermedia patients were found to be statistically significantly higher than the control group. HSP 60 level was found to be 10.1 ± 28.5 in thalassemia major, 2.7 ± 3.1 in thalassemia intermedia and 0.35 ± 0.49 in the control group. Accordingly, a statistically significant difference was detected between the thalassemia major, thalassemia intermedia and control groups in terms of HSP 60. The relationship between thalassemias and HSP 27 has been previously evaluated in two studies. Firstly, in parallel with the results of our study, in a study conducted in Iran with 140 thalassemia major and 140 healthy controls, serum HSP 27 level was significantly higher in the patient group than in the control group [22]. Some of the 64 patients aged between 8 and 18 years were given 30 mg zinc supplements daily, and a significant decrease in HSP 27 levels was detected in the 9th month of the treatment group compared to the pre-treatment period. In the group not given zinc supplementation, an increase in HSP 27 levels was observed at the end of the 9th month, and based on this, it was thought that serum HSP 27 levels could be reduced in thalassemia patients with the potential antioxidant and anti-inflammatory effects of zinc [22].

Limitation

This study has some limitations. First, we did not investigate the potential roles of these proteins in disease progression, hypoxia prediction, or tissue damage assessment. Second, we did not assess disease susceptibility or predict follow-up complications. However, the strength of our study that we established an association between HSP27 and HSP60 levels and general features of thalassemia.

Conclusion

Our study is important as it is the first to measure serum HSP 27 and HSP 60 levels together in thalassemia major and thalassemia intermedia patients, and also to evaluate their relationship with hemogram parameters, liver function tests, ferritin, CRP and splenectomy. Measuring HSP 27 and HSP 60 levels in patients with thalassemia major and thalassemia intermedia can be used as a tool to determine susceptibility to the disease and follow up complications. Our results need to be supported by reproducing similar studies.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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